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**ORIGINAL RESEARCH**

***Reproduction***

**Chatkupt et al. Effect of Continuous Trio Breeding Compared with Continuous Pair Breeding in ‘Shoebox’ Caging on Measures of Reproductive Performance in Estrogen Receptor Knockout Mice, pp. 328-334**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Trio breeding is typically used for rapid colony expansion and to propagate genes from few and/or valuable males. Typical ‘shoebox’ mouse cages are too small for continuous trio breeding (requires: 117 in2) according to the 8th edition of the *Guide*. Reproductive performance is influenced by animal, husbandry and environmental factors. This pilot study compared trio and pair breeding in both wildtype and genetically engineered heterozygous (HET) αβER breeders and their effects on various parameters of reproductive performance. Regardless of genotype, trio breeding had reduced inter-litter interval, but significantly smaller litter sizes at birth and weaning, which may be due to overcrowding and reproductive suppression. HET breeders overall had reduced inter-litter intervals, but reduced weaning rates compared to WT breeders. When breeding, it is important to consider the interactions between genotype and breeding strategy on reproductive performance.

QUESTIONS

1.  Which of the following best describes the estrus cycle of a laboratory mouse?

a.   Seasonal polyestrus, estrus length of 7-8 days

b.   Non-seasonal polyestrus, estrus length 3-4 days

c.   Seasonal polyestrus, estrus length of 3-4 days

d.   Non-seasonal polyestrus, estrus length 7-8 days

2. The 8th edition of the *Guide* states that a female with a litter requires \_\_\_\_\_\_ floor space.

a.  60 in2(387.0 cm2)

b.  51 in2(330 cm2)

c.  15 in2(96.7 cm2)

d.   124 in2 (800 cm2)

ANSWERS

1.  b

2.  b

***Husbandry***

**Hershey et al. Effects of Various Cleaning Agents on the Performance of Mice in Behavioral Assays of Anxiety, pp. 335-339**

Domain 3: Research

Primary Species: Mouse (Mus musculus)

SUMMARY: Behavioral equipment should be cleaned and disinfected between rodent use to prevent disease transmission and reduce odor cues.  The descriptions of cleaning methods in available literature are incomplete and sporadic.  The authors wanted to test various cleaning agents on an elevated plus maze (EPM) to determine if they affect mouse behavior.  C57BL/6J mice were used.

EPM was cleaned with isopropanol, MB10 (200 ppm chlorine dioxide), or bleach (600 ppm), or left unsanitized.  After cleaning, the EPM was wiped and given 30s to dry.  Mouse movement was monitored for 5 minutes.  (10 males, 10 females per group)

* There was no difference between conditions regarding the ratio of entry into open:closed arms of the EPM.
* In all conditions, mice spent more time in closed arms than open.  They spent the most time in unsanitized arms, and the least time in bleach-cleaned arms of the EPM.
* In each condition, female mice entered open areas more often than male mice.

Classic light:dark box.  The dark chamber contained material soaked in one cleaning agent per test.  Mouse movement was monitored for 2 minutes. (8 male mice per group)

* + No statistical difference between groups.  All mice preferred the dark area despite presence of cleaning agent.

Two-choice light:dark box.  One dark chamber contained material soaked in one cleaning agent per test, the other dark chamber’s material was soaked in water.  Mouse movement was monitored for 2 minutes. (8 male mice per group)

* + All three cleaning agents were aversive to mice.  Isopropanol was the most aversive.  The authors suggest the reason this aversion didn’t appear in the EPM test may have been due to the confounding additional stress of a novel device (EPM).

The authors recommend cleaning EPM devices, but perhaps not using isopropanol, due to the aversion seen in the two-choice light:dark box test.  However, note that in the EPM test all cleaning agents were wiped and allowed to evaporate, whereas the light:dark box contained material soaked in each agent.

QUESTIONS

1.  This cleaning agent, when prepared in a strong aqueous solution, will release gaseous chlorine dioxide:

a.  Sodium hypochlorite

b.  Chlorine dioxide

c.  Isopropanol

d.  a and b

e.  b and c

f.  a and c

g.  a, b and c

2.  C57BL/6J mice experience progressive hearing loss.  The mutated allele “age related hearing loss 1” affects what gene?

ANSWERS

1.  d. Sodium hypochlorite and chlorine dioxide.  Gaseous chlorine dioxide may be noxious to personnel.

2.  Cdh23 (cadherin 23)

**Brochu et al. Effects of Nesting Material on the Toxicologic Assessment of Cyclophosphamide in Crl:CD1(ICR) Mice, pp. 340-349**

Primary Species: Mouse (Mus musculus)

Domain 4: Animal Care; K2 - Environmental enrichment

SUMMARY: 48 female and 48 male CD1 outbred stock mice (4-week-old) were split into 4 groups: 1) no nesting material + IP saline 2) 10g nesting material + IP saline 3) no nesting material + IP 10 ml/kg cyclophosphamide 4) 10g nesting material + IP 10 ml/kg cyclophosphamide weekly for 13 weeks. Enviro-dri shepherd specialty paper was provided for nesting material. Results showed that when nesting material was provided, it did not alter hematology, clinical chemistry, B or T cell lymphocyte counts or histopathology findings. Groups with nesting material had significantly lower fecal corticosterone levels which were measured at weeks 1, 2, 6 and 12 suggesting this decreased stress. Cyclophosphamide treated groups had significantly lower nest scores compared to saline treated groups suggesting the general malaise associated with cyclophosphamide treatment resulted in mice not building optimal nests which has been reported as a general indicator of mouse well-being. It was concluded that nesting material had no negative effects on the outcomes of 13 weeks repeated dose cyclophosphamide toxicology study. Overall nesting material decreased stress and helped mice to acclimate faster to injection related stress.

QUESTIONS

1. Which is not an accurate description of the benefits of providing nesting material to rodents?

a. Provides better thermoregulation of environment

b. Improves reproductive performance

c. Improves food conversion

d. Causes an increase in fecal corticosterone levels

e. Increases energy balance during inactivity

2. True or False. Providing enrichment in the form of nesting material for mice is required in the United Kingdom (UK) but not in the United States (US).

3. Mice housed at standard laboratory conditions are cold stressed. What is a mouse’s thermoneutral zone?

1. 20 oC (68 oF)
2. 25 oC (77 oF)
3. 30 oC (86oF)
4. 35 oC (95oF)
5. 40 oC (104 oF)

ANSWERS

1. d. Causes a decrease in fecal cortisone levels
2. True
3. c

**Barone et al. Behavioral Assessment of Vision in Pigs, pp. 350-356**

Primary Species: Pig (Sus scrofa domesticus)

Domain: 3

SUMMARY: Porcine models of retinal eye diseases are becoming popular laboratory animal models to study pathogenesis and therapeutic approaches. Various transgenic pig models of retinal degeneration such as retinitis pigmentosa, Stargardt disease and cone-rod dystrophy have already been developed. In addition, chemical induced model of retinal degeneration using iodoacetic acid which mimics mild to severe form of retinitis pigmentosa is also available for researcher.  Despite the popularity of swine ophthalmology models in retinal diseases, very few available tools that exist to objectively evaluate vision in swine. The full-field flash electroretinography (ffERG), one of most reliable technique used to obtain retinal response, requires anesthesia of the pigs. Reliable and validated behavioral tests could replace ffERG that would provide refinement of the model by avoiding repeated anesthetic procedures. The objective of the current study was to identify and validate a feasible and reproducible behavioral test to subjectively assess vision in a pig model. Using iodoacetic acid-induced photoceptor degeneration swine model in commercial hybrid pig (Large White x Landrace x Durac), authors of the current study evaluated two distinct behavioral tests; obstacle-course test and temperament test. In the obstacle-course test, after initial training period, pigs were challenged to navigate a 10-object obstacle course. Time to complete the course, and the number of collisions with the objects were recorded. In the temperament tests, the time needed for pigs to complete 3 different tasks (human-approach, novel-object, and open-door tests) were recorded. The vision impaired animals (iodoacetic acid-treated animals) took longer time and often collided more with objects under light or dim conditions in the obstacle-course test compared to control animals. However, all temperament tests did not differentiate the control and vision impaired animals. Authors concluded that obstacle-course test can be used objectively to evaluate the vision in swine models of eye disease with prior training period (at least of 3 days) to minimize or avoid any individual animal variabilities. This test is sensitive in detecting differences between the control and iodoacetic acid-treated groups at 3 to 4 wk after treatment, under both light and dim conditions. In contrast, the temperament tests were not sensitive at detecting any vision impairment but these tests are useful to measure stress and behavioral alterations in laboratory pigs. These tests can be used as indicators of habituation and how acquainted these animals to test situations as stress in laboratory animals is known to affect experimental outcomes significantly.

QUESTIONS

1. T/F. Porcine retinal degeneration animal models closely mimic retinal degeneration in human because the retina in pig and humans is similar.

2. Which test that can objectively measure the retinal responses?

3. Which of following can be used to experimentally induced retinal degeneration in pig?

a.   Iodoacetic acid

b.  Acetic acid

c.  4-hydroxytamoxifen

d.  7,12-Dimethylbenz(a)anthracene

ANSWERS

1. True

2. Full-field flash electroretinography

3. a

**Stammen et al. Effect of Chronic Social Stress on Prenatal Transfer of Antitetanus Immunity in Captive Breeding Rhesus Macaques (*Macaca mulatta*), pp. 357-367**

Domain 1

Primary Species: Macaques (Macaque spp)

SUMMARY:

Background: Tetanus is a severe neurologic disease caused by the gram positive obligate anaerobic bacterium *Clostridium tetani*.  In NHP, the most common sources of infection include soil contamination of wounds or postpartum infections.  Clinical diagnosis of tetanus is primarily based on symptoms and vaccination history because *C. tetani* is difficult to culture.  Clinical signs include lethargy, dysphagia, piloerection, and bipedal locomotion characterized by adduction of pectoral limbs with death frequently attributed to respiratory compromise.  Clinical tetanus can cause high morbidity and mortality in NHP, and colony-wide vaccination with tetanus toxoid is recommended for outdoor breeding colonies of rhesus macaques.  Passive immunity against tetanus occurs prenatally through placental transfer.  Infant rhesus macaques are typically vaccinated with tetanus toxoid after 6mths of age to avoid potential interference with maternal antibodies; however, the Yerkes National Primate Research Center diagnosed a large portion of clinical tetanus cases between 0-12mths of age over a 10 year period (24 of 40 documented cases), suggesting that the administration of a single dose of tetanus toxoid to 6-12mth old infants provides insufficient protection.  Previous animal studies suggest that chronic social stress reduces antibody responses to vaccinations.  Because social subordination in female rhesus macaques is a natural chronic psychosocial stressor, the primary aim of the study was to determine whether chronic social stress impairs the prenatal transfer of anti-tetanus immunity in rhesus macaques.  The authors hypothesized that infants born to subordinate dams that were approximately 10yrs after initial immunization would have the lowest infant-to-dam antibody ratios and thus would be at greatest risk of infection.  The authors additionally hypothesized that higher dam hair cortisol levels would predict lower infant-to-dam antibody ratios.

Materials/Methods: Subjects included 52 adult Indian-origin SPF female rhesus macaques and their infants (25 male/27 female) from large outdoor housed breeding groups.  Dams were divided into 2 vaccine groups consisting of 29 dams who were 5 yrs past initial vaccination, and 23 dams who were 10yrs past initial vaccination of tetanus toxoid (TT).  Dam subjects were further designated as either low- or high-ranking based on colony records of overall family rank (Low-5yr, High-5yr, Low-10yr, and High-10yr).  During annual health exams hair samples and 2mL of venous blood were collected from each dam prior to administration of a tetanus booster and 1mL was collected from each infant prior to initial tetanus immunization.  A rhesus-specific TT IgG ELISA was used to measure anti-tetanus IgG levels in the serum with a positive-negative index of 500 U/mL.

Results/Discussion: Significant effects of social rank and vaccination schedule on dam TT IgG levels were found with high-ranking dams exhibiting half the anti-tetanus antibody level of low-ranking dams and lower TT IgG levels in dams 10yrs past initial immunization compared with dams 5 yr past initial immunization (independent of rank).  Notably, the mean TT IgG level of high-ranking dams at 10y after immunization was at the critical positive-negative threshold of 500 U/mL.  Authors hypothesized that anti-tetanus IgG levels were higher in low-ranking dams after accounting for the number of years post-initial vaccination because administration of the primary TT vaccine occurred during annual health examinations, which might be perceived as an acute stressor, particularly by low-ranking animals.  Findings suggest that acute stress has the potential to enhance antibody responses to thymus-dependent vaccines when given in proximity to an acute stressor, especially to subjects with a history of chronic stress exposure.  In contrast and contrary to the primary hypothesis, infant TT IgG levels did not differ significantly among the 4 experimental groups.  However, infant and dam TT IgG levels were strongly correlated.  Analysis revealed a negative relationship between maternal TT IgG levels and the ratio of infant-to-dam IgG levels, suggesting that higher maternal TT IgG levels are associated with less efficient transplacental transfer of TT-specific maternal antibodies to offspring.  Group mean hair cortisol levels were not significantly different among low- and high-ranking dams.  However, maternal hair cortisol was positively related with dam TT IgG levels and negatively correlated with the ratio of infant-to-dam TT IgG levels, demonstrating that chronic stress exposure independently impairs the prenatal transfer of anti-tetanus immunity to the offspring.   The majority of infants in the study between 3 and 6mths of age (regardless of dam’s classification group) had TT IgG levels below the positive-negative threshold, indicating that these infants were not protected against tetanus through maternal antibodies as previously assumed.  The authors recommend that all infants born to dams with any history of a single inoculation should receive their primary TT immunization by 2-3mths of age to ensure full protection.

QUESTIONS

1. Maternal hair cortisol levels were measured to predict which attribute?
2. Chronic stress exposure due to psychosocial stress of subordinate female macaques
3. Acute stress exposure due to psychosocial stress of subordinate female macaques
4. Chronic stress exposure due to psychosocial stress of dominant female macaques
5. Acute stress exposure due to psychosocial stress of dominant female macaques
6. When does IgG transplacental transfer occur in rhesus macaques?
7. 1 to 84d of gestation
8. 84 to 140d of gestation
9. 84 to 164d of gestation
10. 140 to 164d of gestation

ANSWERS

1. a. Maternal hair cortisol levels were measured to provide evidence of a causal relationship between long-term cortisol exposure and stress-induced changes in dam serum anti-tetanus IgG levels and the prenatal transfer of anti-tetanus immunity to offspring.  Because cortisol is incorporated into hair largely through passive diffusion from systemic circulation during the formation of the hair shaft, hair cortisol serves as a noninvasive biomarker of chronic HPA activity and stress exposure over the extended period of time while the hair has been growing.  Subordinate female macaques commonly exhibit HPA dysregulation and have higher basal plasma cortisol levels than their dominant counterparts.
2. c.  In the rhesus macaque, transplacental transfer of IgG starts by day 84 of gestation, with a marked increase in IgG during the last 4 wk of gestation (day 140 through 165).

***Anesthesia***

**Evangelista-Vaz et al. Analgesic Efficacy of Subcutaneous-Oral Dosage of Tramadol after Surgery in C57BL/6J Mice, pp. 368-375**

Domain 2

Primary Species: Mouse (Mus musculus)

SUMMARY: Tramadol is a synthetic opioid used for the treatment of moderate to severe pain in several species. The first and active metabolite of tramadol, O-demethyltramadol, has a 200-fold higher affinity for μ receptors and up to 6-fold higher analgesic potency than tramadol itself. It has been shown that self-administration of tramadol in drinking water results in high and stable plasma levels of drug in naïve C57BL6/J mice throughout consumption time. The authors sought to evaluate the analgesic efficacy of a subcutaneous injection of tramadol (25 mg/kg) followed by voluntary ingestion of tramadol in the drinking water (25 mg/kg) in female C57BL/6J mice undergoing unilateral sham embryo transfer surgery. Four groups of mice (control, tramadol only, anesthesia and surgery only, anesthesia and surgery + tramadol) were evaluated using a pain scoring system (orbital tightening, ear position, abdominal stretch/press, spontaneous movements); automated activity analysis; measurement of body weight, food and water consumption; and latency to burrow test. After a minimum 2-week washout period, 42 mice were reused to establish a pharmacokinetic profile of tramadol. Within 2 hours, serum concentration of tramadol decreased below the established minimum analgesic concentration for humans (100 ng/mL). However, the serum concentration of O-demethyltramadol remained above the human therapeutic level (40 ng/mL) for 18 hours. Composite pain scores were increased in all groups compared with controls, were significantly higher in both surgical groups compared with tramadol only, and did not differ between surgical groups (with and without tramadol). Burrowing latencies were significantly longer in both surgical groups compared with the control group, and latency times were comparable between surgery only and surgery + tramadol groups. Both surgical groups showed decreased activity compared to the control group, but this was only significant for the surgery + tramadol group. Treatment had no effect on water intake (although drinking events appeared reduced in both surgical groups), a significant effect on body weight (between control and surgery + tramadol groups) and a significant effect on food intake (between tramadol only and surgery + tramadol groups. Results indicate that this tramadol dose failed to provide full or sufficient pain relief for mild-to-moderate post-surgical abdominal pain. This may have been due to irregular and variable oral intake, or the dose being too low to reach a therapeutic level for mice. While not significant, composite pain scores were slightly lower for treated compared with untreated operated mice, encouraging further study for more reliable tramadol protocols for mice.

QUESTIONS

1.  Tramadol binds to which opioid receptor(s)?

a.   Mu (μ)

b.  Kappa (κ)

c.   Delta (δ)

d.  κ and δ

e.   μ, κ and δ

2.   Using the mouse grimace scale, what score should this mouse receive for the action unit “orbital tightening”?



a.   0

b.   1

c.  2

d.  5

3.   To deliver a drug dose of ~25 mg/kg daily to a laboratory mouse via its drinking water, what should be the final concentration of the drug in the drinking water? The mouse weighs 25 g.

a.  0.1 mg/mL

b.  1 mg/mL

c.  2.5 mg/mL

d.  10 mg/ mL

e.   25 mg/mL

ANSWERS

1. e

2. c

3. a. Based on water consumption of 6-7 mL/day

**Duval et al. Cardiopulmonary Effects of a Partial Intravenous Anesthesia Technique for Laboratory Swine, pp. 376-381**

Domain 3: Research

Primary Species: Pig (Sus scrofa domesticus)

SUMMARY

Introduction: Limited data on the effects of azaperone in combination with dexmedetomidine and alfaxalone on swine. By using injectable drugs in combination with anesthetic gas can reduce the need for anesthetic gas. Isoflurane and other volatile anesthetics have a dose-dependent effect on hemodynamic and respiratory function of animals and reducing or eliminating their use aids in preserving normal physiologic responses and cardiopulmonary function during general anesthesia. Alfaxalone is a neuroactive steroid and schedule IV drug labeled for the induction and maintenance of anesthesia in cats and dogs.

Materials and Methods: 8 juvenile male landrace-white pigs were obtained from an SPF commercial herd. Animals were premedicated with 0.2mg/kg azaperone, 2.0mg/kg alfaxalone, and 0.02 mg/kg dexmedetomidine. All injections were administered in the epaxial muscles between the shoulder blades. Sedation period was timed from sternal to lateral recumbency. Quality of sedation quantified based on  physical activity, vocalizations, and general behavior.

Anesthesia was induced with alfaxalone to effect (1.4±0.4mg/kg) IV. Bispectral index transcutaneous electrodes (BIS) were placed. Isoflurane was delivered at 40% O@ and 60% medical air with end tidal concentration of 1.2%. Syringe pumps were used to deliver the dexmedetomidine at 2 µg/kg/hr and alfaxalone at 25µg/kg/min IV. NaCl 0.9% given at 5ml/kg/hr was also administered.

Pigs were monitored with a pulse ox, ekg, and capnograph. Surgical cut down with bupivacaine local block to accesses the jugular vein and carotid artery was performed. Thermodilution catheter was inserted into the pulmonary artery. BP, Blood gases, CVP, Pulmonary pressure, and temp was monitored. Thermodilution cath was used to measure CO. Sponge clamp was used on the right dewclaw to assess somatic response to painful stimuli every 10 min. Venous and arterial mixed Blood gases collected every 10 min. Necropsy performed at end of study.

Calculated: systemic vascular resistance: SVR=80 X (MAP-CVP)/CO and Cardiac index (CI): CI=CO(ml/min)/BW(kg)

ANOVA and Mann-Whitney test for Statistical analysis.

Results: 3 out of 8 pigs were underdosed due to incorrect weights. Underdosed animals reacted to insertion of ear cath with mild head shaking other 5 had no response. 7 pigs required total induction dose of 2mg/kg alfaxalone or less. Mean alfaxalone dose for intubation 1.6±0.8mg/kg. 4 pigs had positive responses to claw pinching, 2 animals consistently responded throughout experimental procedure (1 large pig with lowest dose of premed with highest mean BIS number and 1 where premed was sufficient but lowest mean isoflurane over time), 2 pigs responded only once. Mean HR decreased significantly over time at T30 through T60 compared to T0. DAP increased sig over time at T30,40,50,55,60 compared to T0. Pulmonary diastolic and mean varied over time. No gross abnormalities on necropsy

Conclusion: Premedication combination was sufficient for animals to reach lateral recumbency and stay recumbent for transport, IV cath insertion, and physical manipulation. Hypoventilation occurred form T0 to T60 and resulted in mild respiratory acidosis and little change in arterial or venous pH over time. The combination of alfaxalone, dexmedetomidine, and isoflurane caused hypercarbia. The decreased HR over time had minimal clinical effects (diff of 10bpm) with an in in SVR which together was likely caused by the dex. Effects of alfaxalone  on HR are less clear. There was an absence of change in MAP over time and it stayed in the 75-85mmHg. This was expected due to the low dose of each agent. Results were consistent with other studies in dogs, cats, and sheep, thus this protocol could be used on swine.  Did not grade anesthetic depth in the pigs but all were at an adequate plan for the duration of the study. no swallowing or limb movement was observed. BIS values did not increase significantly over time. Further studies need to be done to validate BIS monitoring as a measure of anesthetic depth. The 1 large animal that did not get sufficient premed had a higher BIS score and was suspected to be in a lower anesthetic plane than the other pigs. Reported anesthetic techniques is recommended to minimally invasive diagnostic or therapeutic procedures and additional opioids or local anesthetics are required for more invasive surgical procedures. Overall minimal hemodynamic and pulmonary effects were observed.

QUESTIONS

1. T/F: The dexmedetomidine, alfaxalone, and azaperone combination caused profound changes in the blood pressure and heart rates of the animals

2. What schedule drug in alfaxalone?.

a. I

b. II

c. III

d. IV

e. V

3. How do you calculate systemic vascular resistance?

ANSWERS

1. F

2. d

3.  80 X (MAP-CVP)/CO

***Experimental Use***

**Frohlich et al. Comparison of Serial Blood Collection by Facial Vein and Retrobulbar Methods in C57BL/6 Mice, pp. 382-391**

Domain 3, T1, K1

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Serial blood sample collections are a common occurrence for laboratory mice used for in vivo biomedical research. Retrobulbar (also known as retroorbital) and facial vein are two of the most regularly used techniques. Both collection methods have the potential to cause serious tissue trauma due to the anatomic location of the collection site. To inform institutional policy, this study evaluated the effects of retrobulbar and facial vein techniques when used for serial blood collections in comparison with single sampling. During the 1-h post collection observation period, the serial facial vein group had the most mice with overt clinical adverse effects, followed by serial and single retrobulbar groups, and lastly the single facial vein group. The serial facial vein group had a 33% mortality rate and experienced more serious adverse events. Mice in the facial vein group had significantly greater acute body weight loss compared to mice in the retrobulbar groups. Overall, the current data supports the hypothesis that serial blood collection from mice by using the facial vein method has more adverse outcomes than the retrobulbar method.

QUESTIONS

1.  What gland do you risk damaging when performing the retrobulbar blood collection technique?

2.   What landmarks are used to appropriately perform the facial vein blood collection technique?

ANSWERS

1.       Harderian gland

2.       Approximately 0.5cm dorsal and caudal to the facial scent gland

Primary Species: Mouse (*Mus musculus*)

Domain 3: Research

SUMMARY: Two of the most common blood collection sites for large volume sampling in mice are the retrobulbar/retroorbital area (RB) and the facial vein (FV) but no previous study has compared these two techniques for serial sample collection. Since the FV method collection site is in a large muscle group close to the facial nerve, mandible, temporomandibular joint, and auditory canals, any deviation in the puncture site has the potential to result in adverse bleeding events. The RB method collection site is deep to the ocular globe, which can lead to severe ocular damage if done incorrectly. This study compares the effects of single or serial blood collection by using either the FV or RB blood collection techniques by evaluating CBC, clinical blood chemistry, histopathologic evidence of tissue trauma, and acute change in body weight (BW) and clinical observations of adverse reactions to blood collection. Serial blood collection from mice by using the FV method had more adverse animal health outcomes and greater weight loss than the RB method, with the most severe clinical adverse events of all the groups and a 33% mortality rate. The single FV group had no overt clinical adverse effects. Histologically, blood collection in both the serial FV and RB groups resulted in an increased incidence of tissue damage as compared with their respective single-collection groups. Mice in the single and serial RB groups exhibited similar histologic trauma to the Harderian glands and globe, consistent with previous findings. This study suggests that serial application of the RB method does not dramatically increase the risk for tissue damage. In fact, the most severe histopathologic lesions pertinent to overall animal wellbeing occurred in the serial FV group, in which 16% of animals had evidence of cerebral hemorrhage and ischemia. No differences were detected in clinical chemistries between the single FV and single RB groups or between the serial FV and serial RB groups. This study concludes that the FV method is less desirable for serial blood collection in mice compared with the RB method.

QUESTIONS

1.  For serial blood collection, what is the most desirable site?

a. Facial vein

b. Retrobulbar

c. Cardiac

d. Saphenous vein

2.  **True/False:**Serial blood collection from mice by using the FV method had more adverse animal health outcomes and greater weight loss than the RB method.

3. What is the TNZ for mice, according to the LAM 3rd Ed.?

a.  29.6–30.5°C

b. 30.6–31.5°C

c. 31.6–32.5°C

d. 21.6–33.5°C

ANSWERS

1. b. Retrobulbar

2. True

3. a. 29.6–30.5°C

**Beale et al. Use of Air-activated Thermal Devices during Recovery after Surgery in Mice, pp. 392-400**

Domain 3

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Mice are highly susceptible to hypothermia after anesthetic procedures. Air-activated thermal devices (AATD) are devices which contain mixtures of chemicals that cause an exothermic reaction when exposed to air (i.e. toe warmers which can be used inside boots during cold weather). This experiment tests the ability of AATDs to provide longer term heat support following surgical procedures in mice. They hypothesized that the use of AATDs in static and IVC cages would increase the mouse’s post-operative temperature and decrease the mouse’s time to recovery. They used 6 different cage setups: IVC without AATD, IVC with AATD, static cage on metal surface without AATD, static cage on metal with AATD, static cage on plastic without AATD and static cage on plastic with AATD. They set up cages with the AATD attached to the bottom of the cage in one region. Each mouse was implanted with an intraperitoneal temperature telemetry device which measured their internal temperature following surgery. It was found that temperatures were higher in the IVC cages with AATDs and static cages on plastic surfaces with AATDs. The AATDs were successful in creating a localized area of increased temperature within the cage for 2.5 hours (IVC cages) to 6 hours (static cages) post-surgery. Animals in cages with AATDs did not recover more quickly than the controls and body temperature at 5 hours post op did not differ between the AATD cages and the controls. Mice did seek out the warmer area of the cages with AATDs post operatively. They concluded that AATDs are the most helpful in providing a warmer post-operative environment for mice when placed in static cages on plastic surfaces.

QUESTIONS

1.  True or False. The use of AATDs for post-operative mice was successful in increasing post-operative temperatures as well as decreasing the time to recovery.

2.  AATDs were able to create a warmer localized environment for a longer period of time in which type of cage:

a. IVC cage

b. Static on metal surface

c. Static cage on plastic surface

ANSWERS

1.  False

2.  c

**Del Valle et al. Comparison of Aqueous and Alcohol-based Agents for Presurgical Skin Preparation Methods in Mice, pp. 401-414**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY: The *Guide for the Care and Use of Laboratory Animals* requires the use of aseptic technique for any survival surgeries performed on any laboratory animal species, including rodents.  Components of this technique include appropriate surgeon attire, use of sterile instruments, and appropriate preparation of the surgical site including the application of antiseptic agents.  Aseptic technique reduces the risk of postoperative infection, which can decrease animal welfare by causing animal discomfort and by leading to a breakdown of the surgical site.  Postoperative infection can also negatively affect research parameters of interest by influencing the animal’s physiology.  This study compared various surgical scrub agents, including 70% ethanol, 10% povidone-iodine alternated with saline or 70% ethanol, 2% chlorhexidine digluconate alternated with saline or 70% ethanol, or 1 of 3 waterless alcohol-based (WAB) products (commercial surgical scrub A, commercial surgical scrub B, or a common commercial hand sanitizer) with a 0.9% saline control.  Mice were aseptically prepared for surgical laparotomy using one of test agents, then a 1 cm peritoneal incision through the skin and linea alba was made along the ventral midline mimicking  surgical manipulation during laparotomy.  The povidone-iodine with 70% ethanol demonstrated the greatest average temperature decline during scrub application and the third greatest decline across the entire procedure.  The saline control and povidone-iodine with saline, scrub B, and hand-sanitizer groups demonstrated better heat conservation, with only minimal acceleration of temperature loss over time.  Traditional combination scrub protocols resulted in lower temperatures (greatest risk of hypothermia) than did WAB agents such as scrub B and hand sanitizer, potentially due to fewer applications per mouse.  All 3 novel WAB agents resulted in an absence of detectable bacteria at the surgical site after the recommended number (fewer than 3) applications.  Scrub B and chlorhexidine with 70% ethanol achieved no bacterial presence at the operative site after the surgery was completed, demonstrating persistence of effect.  All WAB agents required fewer steps and time to apply to the operative site.  Scrub B (Avagard Surgical Hand Antiseptic, 61% ethanol and 1% chlorhexidine gluconate) was identified as a WAB skin antiseptic agent comparable to traditional aqueous agents for surgical skin antisepsis and mitigation of body heat loss under anesthesia.

QUESTIONS

1.  Which of the following terms involves the inhibition or prevention of growth of pathogenic microorganisms on living tissue?

a.   Sterilization

b.  Disinfection

c.   Antisepsis

d.   Sanitation

2.  Which of the following agents has fungal and bacterial sporicidal activity in addition to efficacy against gram-positive bacteria, gram-negative bacteria, fungi, and some viruses?

a.   Povidone-iodine

b.   Chlorhexidine

c.  Alcohol

d.  Saline

3.   What can be added to alcohol products to slow their evaporation time?

a.  Analgesic

b.   Emollient

c.  Preservative

d.   Pyridoxine hydrochloride

ANSWERS

1.      c

2.      a

3.      b